Capillary flow of blood in a microchannel with differential wetting for blood plasma separation and on-chip glucose detection

M. Sneha Maria,^{1,2} P. E. Rakesh,¹ T. S. Chandra,² and A. K. Sen^{1,a)}

¹*Department of Mechanical Engineering, Indian Institute of Technology Madras, Chennai 600036, India*

²*Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India*

(Received 9 June 2016; accepted 3 September 2016; published online 22 September 2016)

We report capillary flow of blood in a microchannel with differential wetting for the separation of a plasma from sample blood and subsequent on-chip detection of glucose present in a plasma. A rectangular polydimethylsiloxane microchannel with hydrophilic walls (on three sides) achieved by using oxygen plasma exposure enables capillary flow of blood introduced at the device inlet through the microchannel. A hydrophobic region (on all four sides) in the microchannel impedes the flow of sample blood, and the accumulated blood cells at the region form a filter to facilitate the separation of a plasma. The modified wetting property of the walls and hence the device performance could be retained for a few weeks by covering the channels with deionised water. The effects of the channel cross-section, exposure time, waiting time, and location and length of the hydrophobic region on the volume of the collected plasma are studied. Using a channel cross-section of $1000 \times 400 \mu\text{m}$, an exposure time of 2 min, a waiting time of 10 min, and a hydrophobic region of width 1.0 cm located at 10 mm from the device inlet, 450 nl of plasma was obtained within 15 min. The performance of the device was found to be unaffected (provides 450 nl of plasma in 15 min) even after 15 days. The purification efficiency and plasma recovery of the device were measured and found to be comparable with that obtained using the conventional centrifugation process. Detection of glucose at different concentrations in whole blood of normal and diabetic patients was performed (using $5 \mu\text{l}$ of sample blood within 15 min) to demonstrate the compatibility of the device with integrated detection modules. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4962874>]

I. INTRODUCTION

Diagnostic tests provide critical information about the health status of an individual, thereby helping health care providers and patients to make the right medical decisions. Such tests often provide objective, quantitative measurements that inform every stage of care—prevention, detection, diagnosis, treatment, and successful management of health conditions. Diagnostic test results, including blood tests, inform approximately 70% of medical decisions.¹ Blood is truly a window for the health of the body. Though blood testing is not a substitute for a professional medical diagnosis, it can be used to assess the general state of health, viz., levels of hormones, glucose, minerals, and vitamin deficiencies, confirm the presence of a bacterial or viral infection, inspect organ function, or screen for certain genetic conditions such as cystic fibrosis or spinal muscular atrophy.² Blood, a complex non-Newtonian fluid, is comprised of two components—cells (red blood cells (RBC), white blood cells (WBC), and platelets) suspended in plasma. Cells comprise 45.7% of blood while plasma occupies 54.3% of blood.

^{a)} Author to whom correspondence should be addressed. Electronic mail: ashis@iitm.ac.in

Separation of plasma from blood cells (plasmapheresis) is the prior step for further biochemical analysis³ since most of the blood analyses are based on optical detection techniques and the blood cells need to be removed to decrease the interference with the optical path, thereby increasing assay sensitivity and reliability.⁴

The conventional method of centrifuge based separation of plasma commonly needs milliliters of blood and a labor-intensive handling process in addition to the bulky apparatus. This makes it difficult to conduct self-help and low-cost inspection of drug and other metabolites in blood and hence unsuitable for regular tests on patients, and thus the sample pre-treatment step becomes a bottleneck of the assay process.⁵ Use of microfluidics technology for the sample preparation step would miniaturize and enable easier integration with detection modules. The point-of-care (POC) diagnostic tests will then be made simpler and overcome the difficulties with sample handling, transportation, and storage. Such devices will increase the quality, reproducibility, and reliability of the assay results.³ Extensive reviews are available which detail about various particle separation methods.^{6–14} Also, several works report plasma separation from blood through a variety of techniques. Although active separation methods of plasma separation^{15–19} are efficient in terms of plasma recovery, employment of external fields including electric, magnetic, and acoustic energy limits their portability and cost. Such methods also require longer residence time, which brings down the speed and throughput. Passive methods of plasma separation by making use of the inherent properties of the fluid and the suspended particles are simpler and gentler to the cells. These methods also exhibit high throughput and are cost effective in terms of fabrication, labor, and power.²⁰ The passive separation devices may include filters,^{21,22} microchannel bend structures,^{20,23,24} or microposts²⁵ and require external pressure sources (pumps) for driving the sample fluid, and thus may still be inherent in the challenge of fabrication and operation.²⁶ Therefore, the use of such blood plasma separation devices for point of care diagnostics applications is limited.

As an alternative, self-infusion of a blood sample into a microchannel device using capillary force is more suitable for point-of-care (POC) applications. A summary of the various works reported on a capillary-driven blood plasma separation device is presented in the [supplementary material](#), Table S1. Kim *et al.*²⁶ reported surfactant modified polydimethylsiloxane (PDMS) and SU8 microstructures for driving capillary blood flow and a planar cross-flow filter for plasma separation from whole blood but the plasma recovery was found to be very low (~20 nl plasma) due to the low velocity of the blood flow. Lee and Ahn²⁷ reported a modified surface for achieving plasma separation. It involves successive treatment of Cyclic olefin copolymer (COC) with adhesion promoters and layer by layer coating of five patterned 12 nm silica nanobead structure as superhydrophilic surfaces for creating the asymmetric capillary effect for the pumping and a patch of uncoated area for plasma separation. The plasma recovery of the device is only 6.8%, and the use of silica nanoassembly makes the device fabrication very complicated and expensive. Szydzik *et al.*²⁸ described dielectrophoresis enabled plasma separation from capillary-driven whole blood. Dielectrophoresis forces were used to unblock the filter post structures to increase the extraction of plasma. This device gives better plasma recovery compared with similar works, but the volume of plasma is still less (165 nl), and the micro-electrodes and very shallow plasma extraction channels involve fabrication complexity. Dimov *et al.*⁴ used a filter trench to achieve sedimentation of RBC and a straight microchannel into which the plasma flows from the trench. In addition, a region on the top glass slide overlapping the trench was coated with a hydrophobic pen to improve separation. Son *et al.*²⁹ used a membrane in addition to a vertical up-flow chamber to achieve plasma separation by filtration and sedimentation. Similarly, Kuroda *et al.*³⁰ showed plasma separation with diluted blood (Dilution—1:3) by ascension in a cylindrical channel. But, Dimov *et al.*,⁴ Son *et al.*,²⁹ and Kuroda *et al.*³⁰ used degassing of the channel using a vacuum desiccator to enable the flow of the sample blood into the microchannel device. Although the plasma recovery was found to be higher, the sample blood has to be introduced into the device immediately after the removal of the device from the vacuum. The time gap between the time instants the device is taken out of the vacuum and the sample blood is introduced into the device could affect the efficiency of the device, which limits its use in POC applications.

Here, we present a simple capillary driven PDMS microchannel device with a hydrophobic region for blood plasma separation and detection of glucose in the plasma of normal and diabetic patients. In contrast to other capillary driven blood plasma separation devices reported in the literature, the proposed device is much simpler in terms of design and fabrication and offers higher plasma separation and enhanced purity. The proposed device differs from the device reported by Lee and Ahn,²⁷ in that the device is much simpler in terms of fabrication as opposed to a very complicated procedure (layer by layer coating of a silica nanobead structure) adopted by Lee and Ahn.²⁷ Also, unlike Lee and Ahn, various parameters have been investigated systematically for improving plasma volume, i.e., channel width, length, hydrophilicity levels, patch length, and position. The plasma separation efficiency (22.5%) is higher as compared to that achieved by Lee and Ahn (6.8%). The hydrophilicity of channels was retained by filling with deionised (DI) water, and the separation of plasma in such devices has been reported after 5, 10, and 15 days. The performance of the device was found to be unaffected in terms of plasma volume, separation time, and purification efficiency during this period. First, a brief description of the device and operating principle is presented. Then, a brief theory of capillary flow of blood in hydrophilic and hydrophobic regions is detailed. Further, device fabrication, materials and methods, and the experimental procedure are outlined. Finally, experimental results for blood plasma separation and glucose detection are presented and discussed.

II. DEVICE DESCRIPTION AND PRINCIPLE

A schematic of the proposed capillary driven blood plasma separation device is shown in Fig. 1. The device comprises a PDMS microchannel layer bonded with a PDMS coated glass slide. The PDMS substrate, which forms the top and two side walls of the rectangular microchannel, is hydrophilic everywhere except the hydrophobic region. The PDMS coated glass substrate which forms the bottom wall of the microchannel is hydrophobic throughout. Thus at the hydrophobic region, all four walls of the microchannel are hydrophobic. When the sample blood is introduced at the device inlet, due to the hydrophilic channel walls (on three sides) and thus adequate Young-Laplace pressure, it flows into the microchannel due to capillary action. At the hydrophobic region, due to large contact angles and small Young-Laplace pressure, the motion of the capillary front is impeded. Similar to the phenomenon reported by Lee and Ahn,²⁷ the hydrophobic region acts as a flow barrier, prevents the motion of the blood cells, and leads to the accumulation of blood cells near the region (RBCs adhere to the PDMS wall and accumulate due to the van der Waal interactions^{31,32}). The aggregated blood cells near the hydrophobic region act as a filter to facilitate the separation of plasma. Since the viscosities of the blood cells and plasma are quite different,³³ the blood plasma moves at a higher velocity as compared to the blood cells in the hydrophilic region. Thus while the blood cells stop at the

FIG. 1. Schematic of the capillary driven PDMS microchannel device with a hydrophobic region for the separation of plasma from whole blood and subsequent detection of glucose.

hydrophobic region, due to higher velocity, the blood plasma flows past the hydrophobic region and gets separated from the blood cells.

III. THEORY: CAPILLARY FLOW OF SAMPLE BLOOD

The sample blood flowing in a rectangular microchannel at lower strain rates can be assumed to be non-Newtonian and described using the Casson model³⁴ as follows:

$$\sqrt{\tau} = \sqrt{k_0} + \sqrt{k_1 \dot{\gamma}}, \quad (1)$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, and k_0 and k_1 are constants. Yield stress of blood $k_0 = 0.004$ Pa (Ref. 34) and $k_1 = \eta_p(1 + 0.025H + 7.35 \times 10^{-4}H^2)$, where hematocrit concentration H is taken as 43.8% and viscosity of plasma $\eta_p = 0.012$ Pa s.³⁴ Since the aspect ratio (width:height ratio) of the channel is very high, infinite parallel plate assumption is considered to model the flow. The steady, fully developed Navier-Stokes equation is given as

$$\frac{\partial \tau}{\partial y} = \frac{\partial P}{\partial x}. \quad (2)$$

In the capillary flow situation, the Young-Laplace pressure is the driving pressure, which can be written as

$$P_0 = \sigma \left(\frac{1}{R_1} + \frac{1}{R_2} \right), \quad (3)$$

where σ is the surface tension of blood ($=0.055$ N/m),³⁵ and R_1 and R_2 are the radii of curvature on the top and side walls of the channel. Due to the high aspect ratio of the channel, the radius of curvature along the width is much higher as compared to that along the height. So $(1/R_2)$ is negligible. Therefore, P_0 can be written as

$$P_0 = \frac{2\sigma \cos \theta}{h}, \quad (4)$$

where h is the height of the channel. Since the channel outlet is open to ambient, the pressure gradient along the channel

$$\frac{\partial P}{\partial x} = -\frac{P_0}{x} = C. \quad (5)$$

Next, we substitute the expression for pressure gradient, which is a constant C , from Eq. (5) in Eq. (2) and express shear rate in terms of velocity gradient as $\dot{\gamma} = \partial u / \partial y$. Then, upon integration and by using the boundary conditions, $u = 0$ at $y = 0$ and $\frac{du}{dy} = 0$, $\frac{du}{dy} = 0$ at $y = \frac{h}{2}$, the velocity profile u is obtained as

$$u = \frac{1}{k_1} \left(C \frac{y^2}{2} + \left(2k_0 - C \frac{h}{2} \right) y - \frac{4\sqrt{k_0}}{3C} \left(Cy + k_0 - C \frac{h}{2} \right)^{3/2} + \frac{4\sqrt{k_0}}{3C} \left(k_0 - C \frac{h}{2} \right)^{3/2} \right).$$

Now, the average velocity u_{avg} can be written as

$$u_{avg} = \frac{1}{h} \int_0^h u dy. \quad (6)$$

If we neglect the insignificant terms (and retain only first two terms on the R.H.S.), we get

$$u_{avg} = \frac{1}{k_1} \left(k_0 h + \frac{P_0}{x} \frac{h^2}{12} \right). \quad (7)$$

The above equation gives the expression for the average velocity of the capillary meniscus along the flow direction in the channel.

IV. EXPERIMENTS

A. Device fabrication

The device design was drawn using AutoCAD LT 2008, which was printed on a flexi mask at 40 000 dpi (Fineline Imaging, USA). A silicon wafer (Semiconductor Technology and Application, Milpitas, USA) was cleaned using a hydrofluoric acid (HF) solution and deionised (DI) water at 1:10 and kept in an oven at 120 °C for 2 min. First, SU8 2075 (MicroChem Corp., Newton, USA), a negative photoresist, was spun coated onto the Si wafer with an acceleration of 300 rpm/s. Then, the coated SU8 was soft baked at 65 °C for 3–7 min and hard baked at 95 °C for 6–30 min. Further, the baked SU8 was exposed to UV light (J500-IR/VISIBLE, OAI Mask aligner, CA, USA) through the flexi mask. Next, post-exposure bake of the wafer was performed at 65 °C for 1–5 min and 95 °C at 5–10 min. Then, the pattern was developed by exposing it to SU8 developer and placing inside the oven at 120 °C for 30 min. SU8 master patterns of four different thicknesses, i.e., 50 μm, 100 μm, 200 μm, and 300 μm were fabricated using the process parameters given in [supplementary material](#) Table S2. For the fabrication of the PDMS devices, PDMS monomer and the curing agent (Sylgard 184, Silicone Elastomer kit, Dow Corning, USA) at a ratio of 10:1 (by weight) were mixed and degassed. The mixture was poured onto the SU8 master and then placed in a vacuum oven at 65 °C for 3 h for curing. The baked PDMS was peeled off from the master mold and cut to size. Inlet and outlet holes of around 3 mm diameter were punched using biopsy punches (Shoney Scientific, Pondicherry, India). A small amount of the mixture was also poured onto a glass slide, spin coated (spinNXG-P1, Apex Instruments, India) at 500 rpm for 30 s and baked. The PDMS channel layer was exposed to the oxygen plasma (Harrick Plasma, Brindley St., USA) at power 11 W for 0.5–2 min, while the PDMS coated glass slide was kept unexposed. Finally, the exposed PDMS channel layer and the unexposed PDMS coated glass slide were bonded together by applying a gentle pressure. In experiments employing the hydrophobic region, a thin strip of tape (0.1 mm thick) of required length was placed on the PDMS channel layer across the channel at some distance away from the inlet and then the PDMS channel layer was exposed to oxygen plasma. Thus, a small area of the channel is prevented from getting exposed to the oxygen plasma, which forms the hydrophobic region. Finally, the thin tape was then removed from the PDMS layer and bonded with the PDMS coated glass slide which was not exposed to oxygen plasma. A photograph of the device and an optical image of the microchannel are depicted in [supplementary material](#) Figs. S1(a) and S1(b), respectively.

B. Materials and methods

1. Human blood sample

Samples of human blood from healthy donors were collected from a hospital (Institute Hospital, IIT Madras) in vacutainers with 7.2 mg K2 Ethylene diamine tetraacetic acid (EDTA) (BD, NJ, USA). The blood samples from different diabetic patients were also obtained from our Institute Hospital after ethical clearance and used for the detection experiments.

2. Quantification of the plasma separation

a. Purification efficiency. The purity of the plasma from the reported device and centrifuged blood was compared. For this, the difference in the gray scale intensity of the same section of the channel with and without the plasma was measured in both the cases and compared. Since

the presence of RBCs (darker in colour) in the plasma reduces the gray scale intensity of the channel, a higher difference in the gray scale intensity indicates better purity of a plasma.

b. Plasma recovery. The % plasma recovery was determined by measuring the volumes of plasma and the whole blood present in the channel and using the formula

$$\text{Plasma recovery} = \frac{\text{Volume of plasma separated in the channel}}{\text{Volume of plasma in the whole blood in the channel}} \% \quad (8)$$

C. Experimental setup

A schematic and a photograph of the experimental setup used for the investigation of the blood plasma separation are shown in [supplementary material](#) Figs. S2(a) and S2(b), respectively. A micropipette was used for placing the human blood at the inlet of the microchannel device. The flow of blood inside the channel and the separation of the plasma were observed and captured using an inverted microscope (Carl Zeiss Axiovert A1, Germany) coupled with a high-speed camera (FASTCAM SA3 model, Photron USA, Inc.) interfaced with PC via Photron Fastcam Viewer 3 software.

V. RESULTS AND DISCUSSION

A. Wetting properties of channel walls

Contact angle measurements were performed to demonstrate the effect of oxygen-plasma treatment on the wetting property of PDMS surfaces. The images of DI water and whole blood drops on PDMS surface before and after oxygen-plasma treatment for a duration of 2 min, 11 W, and pressure 460 mTorr at different time instants after the plasma exposure are shown in [supplementary material](#) Figs. S3(a) and S3(b). As shown, the untreated PDMS surface is hydrophobic and the contact angles of water and whole blood drops on untreated PDMS surface are 109° and 98°, respectively. It is observed that when the PDMS surface is exposed to oxygen-plasma, for example, 11 W at 460 mTorr pressure and for a duration of 2 min, the surface is hydrophilic with a contact angle of 38° (water) and 33° (whole blood) even after 3 h. The contact angle of water drops on the treated PDMS surface at 0.5 h, 2 h, and 5.0 h after exposure are found to be 22°, 31°, and 38°, respectively. Similarly, the contact angle of blood drops on the treated PDMS surface at 0.5 h, 2 h, and 5.0 h after exposure are found to be 18°, 31°, and 48°, respectively. The variation of contact angle with waiting time (i.e., time after plasma exposure) for different chamber pressures (fixed exposure time 2 min) is presented in Fig. 2(a). It is observed that at a particular instant of time after plasma exposure, the contact angle is directly proportional to the chamber pressure. This could be because at lower chamber pressure, the mean free path of oxygen molecules is increased thus resulting in a higher momentum loss and consequently limited interaction with the PDMS surface.³⁶ The variation of contact angle of whole blood on the treated PDMS surface with waiting time for different exposure times (fixed chamber pressure 420 mTorr) are presented in Fig. 2(b). As observed, the contact angle decreases when exposure time is increased from 0.5 to 2 min however with a further increase in exposure time to 4 min, the contact angle again decreases. This could be because at much higher exposure times, the continuous oxidation of the PDMS surface leads to the formation of silica layer. Thus, the optimum exposure time of 2 min is used for treating and making the PDMS channel surfaces hydrophilic. It is observed that the contact angle increases with waiting time and the PDMS surface returns back to its native hydrophobicity after approximately 72 h. The return of the plasma exposed PDMS surfaces to its native hydrophobic state can be significantly delayed by keeping the channels soaked inside DI water.³⁷ As shown in Fig. 2(d), after nearly 25 days, the contact angle was found to be approximately 65° indicating that the hydrophilicity of the surface can be maintained for several weeks.

FIG. 2. Variation of contact angle of water on PDMS treated with oxygen plasma at 11 W with (a) different chamber pressures and (b) exposure times. (c) Comparison of contact angle of water and blood. (d) Variation of contact angle of water on PDMS stored immersed in water for retaining hydrophilicity.

B. Capillary flow velocity of whole blood

Simple capillary flow of whole blood in a microchannel with all walls hydrophilic and asymmetric capillary flow of whole blood through a microchannel with top and side walls hydrophilic and bottom wall hydrophobic are presented in Figs. 3(a) and 3(b), respectively. In case of a simple capillary flow, as expected, the meniscus curvature is concave in shape thus the Young-Laplace pressure jump across the meniscus is positive which is responsible for the blood flow. On the other hand, in the case of asymmetric capillary flow, the meniscus appears to be concave in shape only over a very small region closer to the wall and convex in shape over a large region in the channel centre, as shown in Fig. 3(b). The side walls of the channel are hydrophilic and thus the meniscus has concave shape very near the side walls, as expected. The meniscus shape in asymmetric capillary flow was further confirmed using Surface Evolver³⁸ simulations of capillary flow of DI water in a microchannel with three hydrophilic (contact angle 11°) and one hydrophobic (contact angle 109°) walls, as shown in Fig. 3(c). The possible reason behind the convex shape of the meniscus at a region away from the side walls is explained as follows. Since the channel height ($100\ \mu\text{m}$) is much smaller compared to the channel width ($\sim 1000\ \mu\text{m}$), the meniscus curvature in the x - z plane (i.e., R_y) has much higher contribution as compared to that in the x - y plane (i.e., R_z) on the overall pressure jump across the meniscus. Since the meniscus curvature R_y is much smaller at the hydrophobic bottom wall, in order to maintain constant pressure jump $\Delta p = \gamma(1/R_y + 1/R_z)$ across the meniscus throughout, the meniscus has a negative (convex) curvature $-R_z$ over a large region away from the side walls.

FIG. 3. (a) Images showing capillary flow of blood in a microchannel which is fully hydrophilic and (b) asymmetric capillary flow of blood in a microchannel with top and side walls hydrophilic and bottom wall hydrophobic under microscope and (c) in Surface Evolver³⁸ simulation. (d) Comparison of velocity of blood meniscus in symmetric and asymmetric capillary flow.

The variation of normal capillary flow (all walls hydrophilic) and asymmetric capillary flow (top and two sides hydrophilic and bottom hydrophobic) velocity of whole blood along the downstream of a microchannel (size $100 \mu\text{m} \times 1000 \mu\text{m}$) is depicted in Fig. 3(d). In case of a normal capillary flow, the meniscus velocity decreases rapidly from 20 mm/s at the inlet to 0.8 mm/s over a length of 30 mm. On the other hand, in asymmetric capillary flow, the meniscus velocity decreases from 5 mm/s at the inlet to 0.5 mm/s over a distance of 30 mm, at a slower rate as compared to the normal capillary flow case. At any particular location, the meniscus velocity is higher in case of the normal capillary flow as compared to the asymmetric capillary flow case in which one of the channel walls is hydrophobic. The velocity of the capillary blood flow meniscus along the length of the microchannel is predicted using Eq. (7) and validated by comparing with experimental data in case of a microchannel of $100 \times 1000 \mu\text{m}$ size. The experiment was performed after 4 h of plasma exposure when the static contact angle of blood on PDMS is 38° (Fig. 2(c)), and the surface tension of blood is 0.055 N/m.³⁵ The meniscus velocity predicted using the model shows a good agreement with experimental data. The deviation of the model predictions from experimental data could be attributed to the variation in the dynamic contact angle and the constants used in Eq. (7), which also vary with properties of blood and the PDMS surface.

Next, we investigate the effect of waiting time, exposure time, and channel height and width on the meniscus velocity in case of the asymmetric capillary flow. The effect of waiting time (times elapsed after exposure) in the range 10–90 min (for 2 min exposure time) on capillary meniscus velocity along the channel is presented in Fig. 4(b). It is observed that the velocity significantly decreases when waiting time increases from 10 min to 60 min, due to an increase in the contact angle with waiting time (Fig. 2). Although meniscus velocity decreases further with an increase in waiting time, the decrease in velocity from waiting time of 60 min to 90 min is not significant. The slope of the velocity versus distance curve reduces significantly from 10 min to 60 min and decreases slowly with further increase in waiting time. Fig. 4(c)

FIG. 4. (a) Comparison of the velocity of blood meniscus in a microchannel, (b) velocity versus distance for different waiting times for 2 min exposure time, (c) velocity versus distance for three different exposure times at a waiting time of 10 min. (d) Velocity versus distance for different channel height for 2 min exposure time and a waiting time of 10 min.

shows the effect of exposure time (for 10 min waiting time) on the meniscus velocity. As observed, the meniscus velocity increases with an increase in exposure time from 0.5 to 2 min. However, with further increase in exposure time, the meniscus velocity is found to decrease, which is due to the increase in the contact angle beyond an exposure time of 2 min. The effect of channel height on the meniscus velocity is depicted in Fig. 4(d). It is observed that the meniscus velocity increases with an increase in the channel height up to 400 μ m and then reduces with further increase in the channel height. This could be due to the competition between the driving capillary pressure and opposing viscous pressure drop and both the capillary pressure drop and viscous pressure drop decrease with an increase in the channel height. Experiments on effect of channel width on the meniscus velocity showed that the meniscus velocity is almost independent of the channel width in the range of 700–1000 μ m, which is due to the fact that the width of the channel is much higher as compared to the channel height and the effect of the width on the driving pressure is negligible.

C. Blood plasma separation

The optical images of the asymmetric capillary flow meniscus, when it arrives at the hydrophobic region, at various time instants are presented in Fig. 5. The velocity of the capillary flow meniscus along the channel is depicted in Fig. 6(a). It is observed that the meniscus velocity reduces significantly when the meniscus arrives at the hydrophobic region (at $d = 30$ mm). Since the meniscus velocity is very small, the blood cells form a self-built-in filter, which facilitates filtration of blood plasma. Since the separated plasma has a significantly lower viscosity (about 2.5 times) as compared to that of the blood sample, the plasma alone continues to flow downstream. When the meniscus of the separated plasma starts to flow downstream, an increase

FIG. 5. Optical images showing the plasma separation at the hydrophobic region at different time instants.

in the meniscus velocity is observed, as shown in Fig. 6(b). The effect of the length and location of the hydrophobic region, waiting time and the channel height on the volume of the plasma collected is investigated.

Further, experiments were performed to establish optimum length and position of the hydrophobic region. First, 1.0 cm long hydrophobic region was placed at different positions along a 1000 μm wide channel, i.e., between 10 and 50 mm from the device inlet, and volume of plasma after 15 min was measured. As observed in Fig. 7(a), higher plasma volume was obtained when the region is located at <10 mm distance from the inlet. The volume of the separated plasma decreases with an increase in the distance of the hydrophobic region from the device inlet. This is because the meniscus velocity decreases continuously as it flows along the channel (shown in Fig. 4). So when the meniscus moving at a lower velocity stops at the hydrophobic region and the self-built-in filter is formed, the plasma does not have the momentum to pass through the self-built-in filter giving rise to lower plasma separation. The region was not located <10 mm from the device inlet to eliminate the effect of the inlet on the separation process. This indicated the need for a higher meniscus velocity for efficient plasma separation. We performed experiments with a device in which all four channel walls are hydrophilic and a hydrophobic region is located at 10 mm from the device inlet. However, in this case since the meniscus velocity is too high (0.625 cm/s), due to inertia the blood meniscus can easily overcome the hydrophobic region and no plasma separation was observed. So we used our original microchannel device with three hydrophilic and one hydrophobic walls with a hydrophobic region located at 10 mm from device inlet. Next, the effect of the length of the hydrophobic region in the range 0.1–2.0 cm was investigated, and the separated plasma volume obtained

FIG. 6. Variation of (a) blood meniscus velocity along the microchannel with a hydrophobic region and (b) velocity of the separated plasma meniscus in the hydrophobic region.

FIG. 7. Variation of the volume of separated plasma with (a) position of the hydrophobic region, region length 1.0 cm, (b) length of hydrophobic regions, placed at 10 mm from the inlet, (c) waiting times T_w , (d) Channel height.

after 15 min was measured. Fig. 7(b) shows the effect of region length on the volume of the separated plasma. When the region length is too small (i.e., <0.2 cm), plasma separation is only observed for some time but as soon as the plasma meniscus crosses the hydrophobic region, it starts moving fast and pulls the stationary blood cells (and self-built-in filter formed) along with it, thus disturbing the separation process. For regions of length ≥ 0.3 cm, the separation process is not disturbed but the effect of region length on the plasma volume was found to be negligible. In our device, a region length of 1.0 cm was selected for subsequent experiments. A higher region length was avoided in order to reduce the device foot print.

The effect of waiting time (i.e., elapsed time after the oxygen-plasma exposure) on the volume of plasma separation is presented in Fig. 7(c). Here, a channel of 1000 μm width was exposed for 2 min and contains a hydrophobic region of length 1.0 cm at 30 mm from the device inlet. As observed, the volume of plasma decreases with an increase in waiting time which is due to the reduction in meniscus velocity with waiting time (Fig. 4(b)) and thus plasma does not have adequate momentum to pass through the built-in-filter at a higher waiting time. Plasma separation at the region was not observed if the waiting time is more than 1.0 h. However, for practical applications, the plasma separation can be achieved at a longer waiting time by retaining the wetting behaviour of the channels by soaking the devices in DI water, as discussed in Section V A. In an attempt to increase the volume of plasma obtained at 15 min in a 1000 μm wide channel, the effect of different channel heights (i.e., 100–600 μm) was investigated. The effect of channel height on the volume of the separated plasma is depicted in Fig. 7(d). At 15 min, an increase in plasma volume was observed as the height increases upto 400 μm . At 500 μm channel height, the plasma separation decreases and there was no separation

FIG. 8. (a) Comparison of difference in the gray scale intensities (ΔI_g) of a microchannel with and without the presence of a plasma obtained from centrifugation and the proposed device. (b) Comparison of volume of separated plasma with devices stabilized by filling channel with DI water for 5, 10, and 15 days with freshly prepared device. (c) Comparison of difference in the gray scale intensities (ΔI_g) of microchannel with and without the presence of a plasma obtained from centrifugation and the device stabilized in water for 15 days.

observed at $600\ \mu\text{m}$. This may be due to the decrease in the velocity of capillary flow velocity beyond a critical height, i.e., $400\ \mu\text{m}$ resulting in the absence of adequate momentum for the plasma to move past the self-built-in filter. By using a channel height of $400\ \mu\text{m}$, 450 nl of plasma was obtained which is 9-folds higher as compared to that obtained with a channel height of $100\ \mu\text{m}$. Thus, a straight channel of width $1000\ \mu\text{m}$ and height $400\ \mu\text{m}$ with a 1.0 cm long hydrophobic region located at 10 mm from the device inlet was found to be the most favorable design for separating a considerable (i.e., 450 nl) volume of plasma. The proposed device differs from the device reported by Lee and Ahn,²⁷ in that this device does not need successive treatment of adhesion promoters and layer by layer coating of five patterned 12 nm silica nanobead structure for creating the asymmetric capillary effect for the pumping and separation. The proposed device is very simple in terms of fabrication. It only requires exposure to oxygen plasma for making the PDMS surface hydrophilic for the blood flow from the inlet and a tape of required length (for preventing plasma exposure) to create the hydrophobic patch as described under device fabrication (Section IV A) in page 3. Unlike Lee and Ahn, various parameters have been investigated systematically for improving plasma volume, i.e., channel width, length, hydrophilicity levels, patch length, and position. The plasma separation efficiency (22.5%) is higher as compared to that achieved by Lee and Ahn (6.8%).²⁷

In order to test the quality (cell freeness) of the separated plasma, the purification efficiency was determined by measuring the gray scale intensity in a section of channel with and without plasma. Such measurements were performed using the plasma obtained using the proposed device as well as the conventional centrifugation process. Fig. 8(a) shows the comparison of difference of gray scale intensities (ΔI_g) of the plasma obtained using both processes. It was found that the purity of the plasma obtained from the proposed device is higher as compared to that obtained using centrifugation which may be due to the absence of haemolysis which typically happens in centrifugation in the proposed device. In order to demonstrate the stability (retention of hydrophilicity) of the proposed device after two weeks, channels of the fabricated devices (width $1000\ \mu\text{m}$ and height $400\ \mu\text{m}$ with a 1.0 cm long hydrophobic region located at 10 mm from the device inlet) were filled with DI water immediately after oxygen exposure and inlet-outlet ports were sealed using adhesive tapes to prevent evaporation. Separation of a plasma was tested in these devices after 5, 10, and 15 days after removing the DI water from the channel. The performance of the device was found to be unaffected (provides 450 nl of plasma in 15 min) even after 15 days as shown in Fig. 8(b). The purification efficiency was also found to be higher than the centrifugation process similar to the newly prepared device as shown in Fig. 8(c).

D. Detection of glucose

Functionalized surfaces from commercial glucose strips (Accu-Chek Extra Care, Roche Diagnostics India Pvt. Ltd.) were used for the detection of glucose in the plasma separated

FIG. 9. Comparison of difference in the gray scale intensities (ΔI_g) of glucose detection strip on reaction with plasma of non-diabetic and diabetic patients.

from the reported device. Blood samples from diabetic patients were introduced into separate devices. The plasma get separated at the hydrophobic region and flows down stream. The strip whose surface is found to be hydrophilic with a contact angle of 41° is integrated with the channel during bonding (shown in Fig. 1). When the separated plasma comes in contact with the strip, the glucose from blood plasma interacts with the enzyme functionalized surface and depending on the concentration of glucose in plasma, the colour of the strip (originally yellow) changes to different shades of green or blue, which corresponds to specific RGB (Red Green Blue) intensity values. The images of the strips were converted to gray scale, and the gray scale intensities I_g were measured for each sample. The difference between I_g of glucose-reacted and unreacted surface on the strip ΔI_g was used for comparison. The blood samples from diabetic donors were coded (D-1, D-2, and D-3) and compared with ΔI_g measured for the non-diabetic blood sample (C). For validation, the glucose concentration in individual blood samples was obtained with the help of the standard procedure (GOD-POD Enzymatic assay) in our Institute Hospital and the values of C, D-1, D-2, and D-3 were found to be 85, 234, 181, and 141 mg/dl, respectively. The results agree well qualitatively with the ΔI_g measured for the strips treated with the plasma obtained using our device. A correlation between glucose concentration and ΔI_g is observed which shows that ΔI_g is directly proportional to the glucose concentration in blood, as depicted in Fig. 9.

VI. CONCLUSIONS

Development of a self-powered microfluidic device driven by capillary force for the separation of plasma from sample blood is presented and discussed. A theoretical model is developed for predicting the capillary flow velocity of blood along a hydrophilic microchannel and validated. Contact angle measurements and capillary velocity studies were performed to understand the behaviour of blood at different levels of hydrophobicity. The modified wetting property of the PDMS channel surface could be retained for several weeks by filling it with DI water. The contact angle was found to be approximately 65° even after 25 days, indicating that the hydrophilicity of the surface can be maintained. For the separation of plasma, hydrophobic regions were employed and the dimensions of the region and the device were optimized for maximum volume of plasma. A region of length 1.0 cm, placed at 10 mm from the device inlet along the channel of height $400\ \mu\text{m}$ was found to be optimum and enables separation of 450 nl of plasma from whole blood. The plasma obtained was also found to be pure and comparable with that obtained using centrifugation. As compared to reported works on self-actuated devices, higher plasma recovery of 22.5% is obtained using this device. The stability of the proposed device

was demonstrated by performing experiments after two weeks. The performance of the device was found to be unaffected (provides 450 nl of plasma in 15 min) even after 15 days. Detection of glucose proves the suitability of the plasma for ensuing analytic tests.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for a summary of work reported in the literature on capillary based blood plasma separation, process parameters used for channel fabrication, a photograph of the cell-plasma separation device, experimental setup, and images showing water and blood droplets on the PDMS surfaces.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Biotechnology, India (BT/PR7276/MED/32/267/2012), Department of Science and Technology, India (EMR/2014/001151), and I.I.T. Madras (MEE15-16843RFTPASHS) for providing the financial support for the project. We also acknowledge CNRP, IIT Madras for supporting the photolithography work. We also thank Department of Applied Mechanics, IIT Madras, for the contact angle measurement facility. Our special thanks to Interdisciplinary Program, IIT Madras which enabled this work.

¹The Essentials of Diagnostics whitepaper, DX Insights, January 2012.

²See <http://www.loughtonclinic.org/blood-testing.html> for the uses of blood test.

³M. L. Kovarik, P. C. Gach, D. M. Orloff, Y. Wang, J. Balowski, L. Farrag, and N. L. Allbritton, "Micro total analysis systems for cell biology and biochemical assays," *Anal. Chem.* **84**(2), 516–540 (2012).

⁴I. K. Dimov, L. Basabe-Desmonts, J. L. Garcia-Cordero, B. M. Ross, A. J. Ricco, and L. P. Lee, "Stand-alone self-powered integrated microfluidic blood analysis system (SIMBAS)," *Lab Chip* **11**, 845–850 (2011).

⁵X. Zhang, Z. Wu, K. Wang, J. Zhu, J. Xu, X. Xia, and H. Chen, "Gravitational sedimentation induced blood delamination for continuous plasma separation on a microfluidics chip," *Anal. Chem.* **84**(8), 3780–3786 (2012).

⁶H. W. Hou, A. A. S. Bhagat, W. C. Lee, S. Huang, J. Han, and C. T. Lim, "Microfluidic devices for blood fractionation," *Micromachines* **2**, 319–343 (2011).

⁷D. R. Gossett, W. M. Weaver, A. J. Mach, S. C. Hur, H. T. K. Tse, W. Lee, H. Amini, and D. Di Carlo, "Label-free cell separation and sorting in microfluidic systems," *Anal. Bioanal. Chem.* **397**, 3249–3267 (2010).

⁸M. Kersaudy-Kerhoas and E. Sollier, "Micro-scale blood plasma separation: From acoustophoresis to egg-beaters," *Lab Chip* **13**, 3323–3346 (2013).

⁹N. Pamme, "Continuous flow separations in microfluidic devices," *Lab Chip* **7**, 1644–1659 (2007).

¹⁰P. Sajeesh and A. K. Sen, "Particle separation and sorting in microfluidic devices: A review," *Microfluid. Nanofluid.* **17**, 1–52 (2014).

¹¹M. Toner and D. Irimia, "Blood-on-a-chip," *Annu. Rev. Biomed. Eng.* **7**, 77–103 (2005).

¹²A. A. S. Bhagat, H. Bow, H. W. Hou, S. J. Tan, J. Han, and C. T. Lim, "Microfluidics for cell separation," *Med. Biol. Eng. Comput.* **48**, 999–1014 (2010).

¹³M. Kersaudy-Kerhoas, R. Dhariwal, and M. P. Y. Desmulliez, "Recent advances in microparticle continuous separation," *IET Nanobiotechnol.* **2**(1), 1–13 (2008).

¹⁴A. Lenshof and T. Laurell, "Continuous separation of cells and particles in microfluidic systems," *Chem. Soc. Rev.* **39**, 1203–1217 (2010).

¹⁵L. Y. Yeo, J. R. Friend, and D. R. Arifin, "Electric tempest in a teacup: The tea leaf analogy to microfluidic blood plasma separation," *Appl. Phys. Lett.* **89**, 103516–1–3 (2006).

¹⁶Y. Nakashima, S. Hata, and T. Yasuda, "Blood plasma separation and extraction from a minute amount of blood using dielectrophoretic and capillary forces," *Sens. Actuators, B* **145**, 561–569 (2010).

¹⁷H. Jiang, X. Weng, C. H. Chon, X. Wu, and D. Li, "A microfluidic chip for blood plasma separation using electro-osmotic flow control," *J. Micromech. Microeng.* **21**, 085019–1–8 (2011).

¹⁸A. Lenshof, A. Ahmad-Tajudin, K. Järås, A. Swärd-Nilsson, L. Åberg, G. Marko-Varga, J. Malm, H. Lilja, and T. Laurell, "Acoustic whole blood plasmapheresis chip for prostate specific antigen microarray diagnostics," *Anal. Chem.* **81**, 6030–6037 (2009).

¹⁹E. P. Furlani, "Magnetophoretic separation of blood cells at the microscale," *J. Phys. D: Appl. Phys.* **40**, 1313–1319 (2007).

²⁰M. S. Maria, B. S. Kumar, T. S. Chandra, and A. K. Sen, "Development of a microfluidic device for cell concentration and blood cell-plasma separation," *Biomed. Microdevices* **17**, 115 (2015).

²¹T. A. Crowley and V. Pizziconi, "Isolation of plasma from whole blood using planar microfilters for lab-on-a-chip applications," *Lab Chip* **5**, 922–929 (2005).

²²K. Aran, A. Fok, L. A. Sasso, N. Kamdar, Y. Guan, Q. Sun, A. Undar, and J. D. Zahn, "Microfiltration platform for continuous blood plasma protein extraction from whole blood during cardiac surgery," *Lab Chip* **11**, 2858–2868 (2011).

²³C. Blattert, R. Jurischka, A. Schoth, P. Kerth, and W. Menz, "Separation of blood cells and plasma in microchannel bend structures," *Lab Chip: Plat. Dev. Appl.* **5591**, 143–151 (2004).

²⁴M. Faivre, M. Abkarian, K. Bickraj, and H. A. Stone, "Geometrical focusing of cells in a microfluidic device: an approach to separate blood plasma," *Biorheology* **43**, 147–159 (2006).

- ²⁵J. A. Davis, D. W. Inglis, K. J. Morton, D. A. Lawrence, L. R. Huang, S. Y. Chou, J. C. Sturm, and R. H. Austin, "Deterministic hydrodynamics: Taking blood apart," *Proc. Natl. Acad. Sci. U.S.A.* **103**, 14779–14784 (2006).
- ²⁶Y. C. Kim, S. Kim, D. Kim, S. Park, and J. Park, "Plasma extraction in a capillary-driven microfluidic device using surfactant-added poly(dimethylsiloxane)," *Sens. Actuators, B* **145**(2), 861–868 (2010).
- ²⁷K. K. Lee and C. H. Ahn, "A new on-chip whole blood/plasma separator driven by asymmetric capillary forces," *Lab Chip* **13**(16), 3261–3267 (2013).
- ²⁸C. Szydzik, K. Khoshmanesh, A. Mitchell, and C. Karnutsch, "Microfluidic platform for separation and extraction of plasma from whole blood using dielectrophoresis," *Biomicrofluidics* **9**, 064120 (2015).
- ²⁹J. H. Son, S. H. Lee, S. Hong, S. Park, J. Lee, A. M. Dickey, and L. P. Lee, "Hemolysis-free blood plasma separation Hemolysis-free blood plasma separation," *Lab Chip* **14**, 2287 (2014).
- ³⁰C. Kuroda, Y. Ohki, H. Ashiba, M. Fujimaki, K. Awazu, T. Tanaka, and M. Makishima, "Microfluidic sedimentation system for separation of plasma from whole blood," in *2014 IEEE Sensors* (IEEE, 2014).
- ³¹E. M. Keough, W. C. Mackey, R. Connolly, T. Foxall, K. Ramberg-Laskaris, J. L. McCullough, T. F. O'Donnell, Jr., and A. D. Callow, "The interaction of blood components with PDMS (polydimethylsiloxane) and LDPE (low-density polyethylene) in a baboon ex vivo arteriovenous shunt model," *J. Biomed. Mater. Res.* **19**(5), 577–587 (1985).
- ³²K. Kendall and A. D. Roberts, "van der Waals forces influencing adhesion of cells," *Philos. Trans. R. Soc. B* **370**, 20140078 (2015).
- ³³O. Linderkamp, H. T. Versmold, K. P. Riegel, and K. Betke, "Contributions of red cells and plasma to blood viscosity in preterm and full-term infants and adults," *Pediatrics* **74**(1), 45–51 (1984).
- ³⁴E. W. Merrill, "Rheology of blood," *Physiol. Rev.* **49**(4), 863–888 (1969).
- ³⁵J. Rosina, E. Kvašňák, D. Šuta, H. Kolářová, and J. Málek, "Temperature dependence of blood surface tension," *Physiol. Res.* **56**, S93–S98 (2007).
- ³⁶S. Bhattacharya, A. Datta, J. M. Berg, and S. Gangopadhyay, "Studies on surface wettability of poly(dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength," *J. Microelectromech. Syst.* **14**(3), 590–597 (2005).
- ³⁷S. H. Tan, N. Nguyen, Y. C. Chua, and T. G. Kang, "Oxygen plasma treatment for reducing hydrophobicity of a sealed polydimethylsiloxane microchannel," *Biomicrofluidics* **4**, 032204 (2010).
- ³⁸K. Brakke, "The surface evolver (PDF)," *Exp. Math.* **1**(2), 141–165 (1992).